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March 3, 2008  
David R. Saliwanchik  
David R. Saliwanchik, Patent Attorney

REQUEST FOR CERTIFICATE OF  
CORRECTION UNDER 37 CFR 1.322  
Docket No. CHROM-3XC1  
Patent No. 7,317,111

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Ram Bhatt, Michael J. Conrad, Azzouz Bencheikh, Yifeng Xiong  
Issued : January 8, 2008  
Patent No. : 7,317,111  
For : Novel Green and Orange Fluorescent Labels and Their Uses

Mail Stop Certificate of Corrections Branch  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION  
UNDER 37 CFR 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

**Patent Reads:**

Cover Page, Item 75, Lines 3-4:

"San Diego, CA"

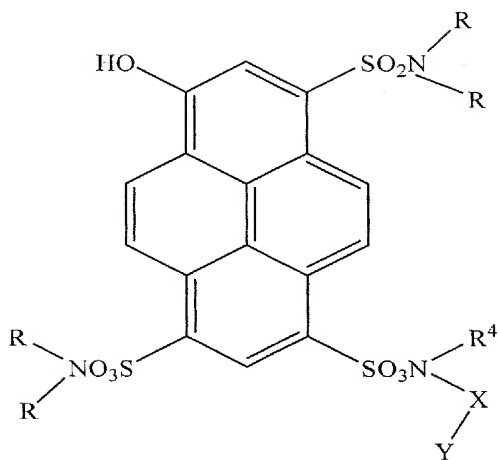
**Application Reads:**

Page 3 of the Declaration under 37 CFR 1.63  
and Power of Attorney ("Residence" for  
"Azzouz Bencheikh"):

--Rancho Santa Fe, California--

**Patent Reads:**Column 11, Line 30:

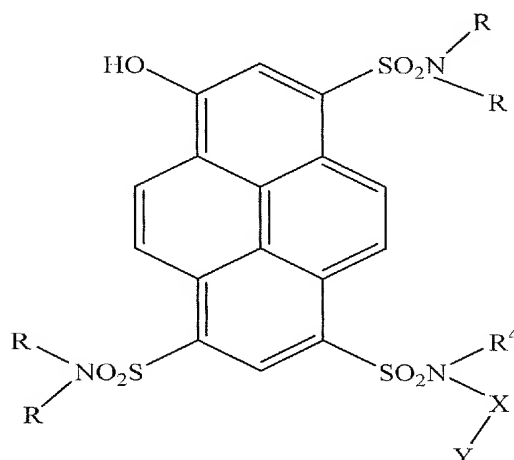
"



"

**Application Reads:**Page 16 of the Specification, Structure II :

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Column 11, Table 1, Line 58:"-SH, -NH<sub>2</sub>, -NCS, -NCO,."Page 17 of the Specification, Table 1, Column R2:---SH, -NH<sub>2</sub>, -NCS, -NCO,--Column 19, Line 41:

"λ ex = 490 nm"

Page 28 of the Specification, Line 23:

--λ ex = 490 nm--

Column 23, Line 23:

"GCT.GCT.GCA.GGT.CGA.GAA.GGC.TTC"

Page 33 of the Specification, Line 20:

--GCT.GCA.GGT.CGA.GAA.GGC.TTC--

True and correct copies of the Declaration under 37 CFR 1.63 and Power of Attorney and pages 16, 17, 28, and 33 of the specification as filed which support Applicants' assertion of the errors on the part of the Patent Office accompanies this Certificate of Correction.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,



David R. Saliwanchik  
Patent Attorney  
Registration No. 31,794  
Phone No.: 352-375-8100  
Fax No.: 352-372-5800  
Address: P.O. Box 142950  
Gainesville, FL 32614-2950

DRS/yvs

Attachments: Certificate of Correction  
Declaration under 37 CFR 1.63 and Power of Attorney  
Pages 16, 17, 28, and 33 of the specification as filed

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,317,111

Page 1 of 2

DATED : January 8, 2008

INVENTORS : Ram Bhatt, Michael J. Conrad, Azzouz Bencheikh, Yifeng Xiong

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Cover Page,

Item 75, Lines 3-4, "San Diego, CA" should read --Rancho Santa Fe, CA--.

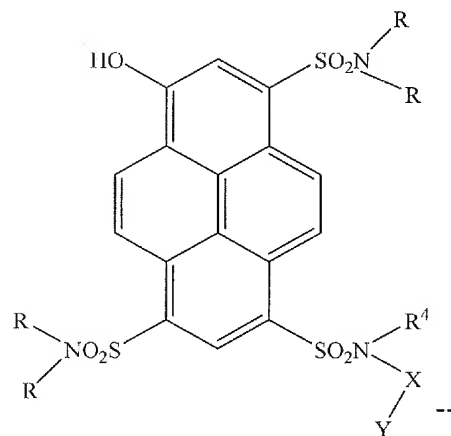
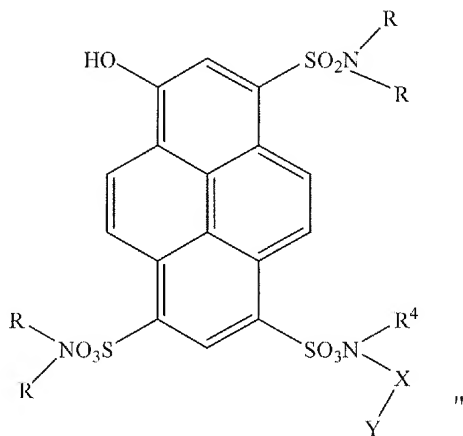
Column 11,

Line 30,

"

should read:

--



MAILING ADDRESS OF SENDER:

Saliwanchik, Lloyd & Saliwanchik  
P.O. Box 142950  
Gainesville, FL 32614-2950

Patent No. 7,317,111

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,317,111

Page 2 of 2

DATED : January 8, 2008

INVENTORS : Ram Bhatt, Michael J. Conrad, Azzouz Bencheikh, Yifeng Xiong

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 11.

Table 1, Line 58, "-SH, -NH<sub>2</sub>, -NCS, -NCO,." should read ---SH, -NH<sub>2</sub>, -NCS, -NCO,--.

Column 19.

Line 41, "λ ex = 490 run" should read --λ ex = 490 nm--.

Column 23.

Line 23, "GCT.GCT.GCA.GGT.CGA.GAA.GGC.TTC" should read  
--GCT.GCA.GGT.CGA.GAA.GGC.TTC--.

MAILING ADDRESS OF SENDER:  
Saliwanchik, Lloyd & Saliwanchik  
P.O. Box 142950  
Gainesville, FL 32614-2950

Patent No. 7,317,111

Please type a plus sign (+) inside this box → ☐

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Approved for use through 10/31/2002. OMB 0651-0032

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# UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No. CHROM-3XC1  
First Inventor Ram Bhatt  
Title Novel Green and Orange Fluorescent Labels and Their Uses  
Express Mail Label No. EU 082848728 US

## APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. ☒ Fee Transmittal Form (e.g., PTO/SB/17)  
(Submit an original and a duplicate for fee processing)
2. ☒ Applicant claims small entity status.  
See 37 CFR 1.27.
3. ☒ Specification [Total Pages 56]  
(preferred arrangement set forth below)
  - Descriptive title of the invention
  - Cross Reference to Related Applications
  - Statement Regarding Fed sponsored R & D
  - Reference to sequence listing, a table, or a computer program listing appendix
  - Background of the Invention
  - Brief Summary of the Invention
  - Brief Description of the Drawings (if filed)
  - Detailed Description
  - Claim(s)
  - Abstract of the Disclosure
4. ☒ Drawing(s) (35 U.S.C. 113) [Total Sheets 23]
5. ☒ Oath or Declaration [Total Pages 3]
  - a. ☐ Newly executed (original or copy)
  - b. ☒ Copy from a prior application (37 CFR 1.63 (d))  
(for continuation/divisional with Box 18 completed)
    - i. ☐ **DELETION OF INVENTOR(S)**  
Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).
6. ☐ Application Data Sheet. See 37 CFR 1.76

ADDRESS TO: Assistant Commissioner for Patents  
Box Patent Application  
Washington, DC 20231

7. ☐ CD-ROM or CD-R in duplicate, large table or Computer Program (Appendix)
8. Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary)
  - a. ☐ Computer Readable Form (CRF)
  - b. Specification Sequence Listing on:
    - i. ☐ CD-ROM or CD-R (2 copies); or
    - ii. ☐ paper
  - c. ☐ Statements verifying identity of above copies

## ACCOMPANYING APPLICATION PARTS

9. ☐ Assignment Papers (cover sheet & document(s))
10. ☐ 37 CFR 3.73(b) Statement of Power of Attorney (when there is an assignee)
11. ☐ English Translation Document (if applicable)
12. ☐ Information Disclosure Statement (IDS)/PTO-1449
13. ☐ Preliminary Amendment
14. ☒ Return Receipt Postcard (MPEP 503) (Should be specifically itemized)
15. ☐ Certified Copy of Priority Document(s) (if foreign priority is claimed)
16. ☐ Request and Certification under 35 U.S.C. 122 (b)(2)(B)(i). Applicant must attach form PTO/SB/35 or its equivalent.
17. ☒ Other: Certificate of Mailing by Express Mail

18. If a CONTINUING APPLICATION, check appropriate box, and supply the requisite information below and in a preliminary amendment, or in an Application Data Sheet under 37 CFR 1.76:

☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No.: \_\_\_\_\_

Prior application information: Examiner \_\_\_\_\_ Group Art Unit: \_\_\_\_\_

For CONTINUATION OR DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 5b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

## 19. CORRESPONDENCE ADDRESS

☒ Customer Number or Bar Code Label 23,557 or ☐ Correspondence address below

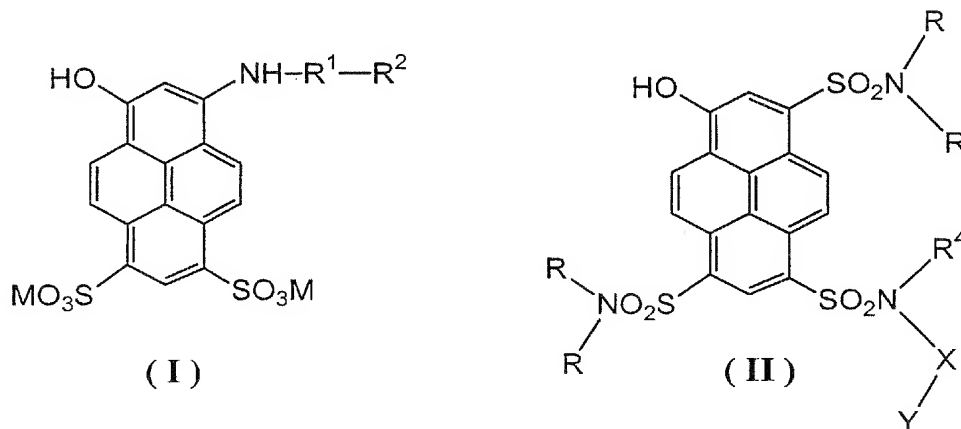
Name \_\_\_\_\_  
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Country \_\_\_\_\_ Telephone \_\_\_\_\_ Fax \_\_\_\_\_

Name (Print/Type) Margaret Efron Registration No. (Attorney/Agent) 47,545  
Signature \_\_\_\_\_ Date 09/23/03

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their distal ends and through which the fluorophore can be covalently attached as a fluorescent label to another, non-fluorescent molecule such as a protein, oligonucleotide, lipid, small molecule ligand or a peptide.

5 In a preferred embodiment, the substrates of the subject invention have the general formula represented by the structures (I) or (II) :



10 where R<sup>1</sup>, R<sup>2</sup>, X and Y represent spacers and conjugating linkers of different compositions, and where R and R<sup>4</sup> are hydrogen, alkyl or phenyl substituents. Specific examples are shown in the following tables.

Table 1. Spacers and Linkers associated with the compounds of Structure I	
R1	R2
CO – (CH <sub>2</sub> ) <sub>n</sub> where n = 1-15	- COOH, - SH, -NH <sub>2</sub> , -NCS, -NCO, - CO <sub>2</sub> -NHS, - Maleimide
- CO- PEG	- COOH, - SH, -NH <sub>2</sub> , -NCS, -NCO, - CO <sub>2</sub> -NHS, - Maleimide
- CO- DEXTRAN	- COOH, - SH, -NH <sub>2</sub> , -NCS, -NCO, - CO <sub>2</sub> -NHS, - Maleimide
CO(CH <sub>2</sub> ) <sub>n</sub> -(CONHCHCONH) <sub>N</sub> <div style="text-align: center;">                R     </div> n = 1-15      R = Alkyl, Aryl N = 1 -100	- COOH, - SH, -NH <sub>2</sub> , -NCS, -NCO, - CO <sub>2</sub> -NHS, - Maleimide
CO- Aryl-(CH <sub>2</sub> ) <sub>n</sub> n= 1-15	- COOH, - SH, -NH <sub>2</sub> , -NCS, -NCO, - CO <sub>2</sub> -NHS, - Maleimide
CO (CH <sub>2</sub> ) <sub>n</sub> – CONH-(CH <sub>2</sub> ) <sub>N</sub> - n= 1-15, N= 1-15	- SH, -NH <sub>2</sub> , -NCS, -NCO, - Maleimide, -NHNH <sub>2</sub>
CO (CH <sub>2</sub> ) <sub>n</sub> - CONH- PEG n = 1-15	- SH, -NH <sub>2</sub> , -NCS, -NCO, - Maleimide, -NHNH <sub>2</sub>
CO (CH <sub>2</sub> ) <sub>n</sub> - CONH- DEXTRAN n = 1-15	- SH, -NH <sub>2</sub> , -NCS, -NCO, - Maleimide, -NHNH <sub>2</sub>
CH <sub>2</sub> - (CH <sub>2</sub> ) <sub>n</sub> - CONH- X Where X = (CH <sub>2</sub> ) <sub>n</sub> , n = 1-15 = PEG = Dextran CH <sub>2</sub> -(CH <sub>2</sub> ) <sub>n</sub>	- SH, -NH <sub>2</sub> , -NCS, -NCO, COONHS, - Maleimide, -NHNH <sub>2</sub>
CH <sub>2</sub> -(CH <sub>2</sub> ) <sub>n</sub> n = 1-15	- SH, -NH <sub>2</sub> , -NCS, - Maleimide, COONHS

Example 11 — 1-Hydroxy-3,6-di-(dimethylsulfonamido)-8-(3-aminopropyl-methylsulfonamido)-pyrene ( compound 15 )

To chlorosulfonic acid (8 ml) was added compound **14** (940 mg, 1.83 mMol) at room temperature. The mixture was stirred at room temperature over the weekend and quenched with ice carefully. The solid was filtered off, dried on the vacuum for 30 min. This was added to a solution of N-methyl-3-amino-propane (2M in THF, 20 ml) in acetone (5 ml). The resulting mixture was stirred for 1 h and concentrated in *vacuo*. Flash chromatography on a silica gel column using CHCl<sub>3</sub>-MeOH (4:1) provided the desired product **15** as a yellow solid (540 mg, 50%). <sup>1</sup>H NMR (DMSO, 500 mHz): 1.85-1.90 (m, 2H), 2.65-2.83 (m, 12H), 2.89 (s, 3H), 3.30-3.36 (m, 6H), 8.0 (s, 1H), 8.25-8.28 (m, 1H), 8.80-8.90 (m, 3H), 8.97-9.0 (m, 1H); MS (M-H): 581; fluorescence (MeOH/H<sub>2</sub>O): λ<sub>ex</sub> = 490 nm, λ<sub>em</sub> = 548 nm.

Example 12 — 1-Hydroxypyrene-3,6-di-(dimethylsulfonamido)-8-(3-isothiocyanatopropyl-methylsulfonamide ( compound 16 )

To a solution of **15** (230 mg, 0.40 mMol) in DMF (8 ml) was added thiocarbonyl diimidazole (142 mg, 0.8 mMol). The mixture was stirred at room temperature for a few hours and concentrated in *vacuo*. Flash chromatography on silica gel column, as described above, provided StarBright **Orange**, compound **16** as a yellow solid (200 mg, 80%). <sup>1</sup>H NMR (DMSO, 500 mHz): 1.89 (t, 2H), 2.81-2.84 (m, 12H), 2.86 (s, 3H), 3.27 (t, 2H), 3.66 (t, 2H), 8.33 (s, 1H), 8.85 (d, 1H), 9.0 (s, 1H), 9.05 (d, 1H), 9.14 (d, 1H), 9.20 (d, 1H), 12.0 (s, br, 1H); MS (M-H): 623; fluorescence (MeOH/H<sub>2</sub>O): λ<sub>ex</sub> = 490 nm, λ<sub>em</sub> = 548 nm.

Example 13 — 1-Hydroxy-3,6-di(dimethylsulfonamido)-8-(4'-succinimidylbutyrate)-phenylsulfonamido- pyrene ( compound 17 )

Compound **14** ( 550 mg, 1.07 mMol) was dissolved in chlorosulfonic acid (10 ml) and stirred at room temperature overnight. The resulting sulfonyl chloride was then precipitated on ice and the solid filtered off and washed with 2x 20 ml of cold water. This precipitated was then re-dissolved in 40 ml of dry THF and treated dropwise with a THF (10ml) solution of 4-aminophenyl Butyric acid (283 mg 2.14 mMol). The resulting dark orange solution was stirred at room temperature overnight

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The complete structure of SBG as attached to an oligonucleotide is shown below:



25

The MMT-NH<sub>2</sub>-oligonucleotide was treated with 80% acetic acid in water at RT for 1 hour to remove the MMT group to obtain free amino group at the distal end of the linker attached to the 5'-end of the oligonucleotide. After evaporating the acetic acid